REMARKS/ARGUMENTS

The claims of this application are directed to the non-elected claims of the parent application.

The disclosure has been revised to introduce a Sequence Listing, correct clerical errors and to correct the designation of the Figures to correspond to the formal drawings submitted.

These changes are made at the present time in the interests of providing a specification in better form for examination.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**."

It is believed that this application is now in condition for allowance and early and favourable consideration and allowance are respectfully solicited.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 11 of page 7 has been amended as follows:

Figures 2A and 2B[, comprising panels A and B,] illustrate[s] protection against MoPn infection with *momp* gene products following DNA immunization. Balb/c mice were immunized with (o) pcDNA3 (n = 11), (•) pMOMP intramuscularly (n = 12), (Δ) pMOMP intranasally (n = 5) or (▲) MoPn EBs (n = 12). Eighteen days after the last immunization, mice were challenged intranasally with infectious MoPn (1000 IFU). Figure 2A [Panel A] shows body weight loss. Body weight was measured daily following infection challenge and each point in Figure 2A[, panel A], represents the mean ± SEM of the body weight loss. [Panel]Figures 2B shows *in vivo* chlamydia clearance. Mice were sacrificed day 10 postinfection and recovery of infectious MoPn from lung tissue was analyzed by quantitative tissue culture in order to determine the *in vivo* chlamydial clearance. The data in Figure 2B[, panel B], represent mean ± SEM of the $\log_{10}$ IFU per lung.

Paragraph beginning at line 3 of page 8 has been amended as follows:

Figures 4A, 4B, 4C and 4D[, comprising panels A, B, C and D,] compare[s] serum 1gG subclasses $1gG_{2a}$ ([Panels A and C]Figures 4A and 4C) with IgG, [Panels B and D] Figures 4B and 4D) against recombinant MOMP protein [(Panel]Figures 4A and 4B) or MoPn EBs ([Panel]Figures 4C and 4D) induced by DNA immunization. Mice were non-immunized or immunized intramuscularly with pMOMP, CTP synthetase DNA (pCTP) or the blank plasmid vector (pcDNA3) at 0,3,6 weeks and pooled sera from each group were collected two weeks following the last immunization (day 10). The data in Figures 4A and 4B represent mean ± SEM of the OD value of four duplicates.

Paragraph beginning at line 14 of page 8 has been amended as follows:

Figures 5A and 5B[, comprising panels A and B,] demonstrate[s] that DNA vaccination with the MOMP gene enhanced clearance of MoPn infection in the lung. Groups of Balb/c mice were immunized with pMOMP (n = 10), pcDNA3 (n = 10) or saline (n = 5). Eighteen days after the last immunization, the mice were challenged intranasally with infectious MoPn (10^4 IFU). [Panel]Figure 5A shows the body weight of the mice measured daily following challenge infection until the mice were sacrificed at day 10. Each point in Figure 5[, panel]A, represents the mean ± SEM of the body weight change. * represents $P < .05$ compared with pcDNA3 treated group. [Panel]Figure 5B: the mice were sacrificed at day 10 postinfection and the MoPn growth in the lung was analyzed by quantitative tissue culture. The data in Figure 5[, panel]B, represent mean ± SEM of the Log_{10}IFU per lung. * represents $P < .01$ compared with pcDNA3 treated group.

Paragraph beginning at line 31 of page 8 has been amended as follows:

Figures 6 A and 6B show[, comprising panels A and B, shows] evaluation of the responses of mice to MoPn intranasal challenge infection. Figure 6A[Panel] shows the change in body weight post challenge and Figure 6B[Panel] shows the growth of MoPn in lung tissue collected 10 days after challenge. Mice were sham immunized, immunized intraperitoneally with MoPn EBs recovered from prior MoPn lung infection, or immunized intramuscularly with p½MOMP. * represents $P<10^{-3}$ compared to the pcDNA3 treated group. ** represents $P<10^{-4}$ compared to the pcDNA3 treated group.

Paragraph beginning at line 26 of page 11 has been amended as follows:

Figures 20A to 20F show[s] a comparison of the amino acid sequence of MOMP sequences (SEQ ID NOS: 1 to 15) from a variety of serovars of *C. trachomatis*. Residues which are identical to serovar E MOMP are represented by dots. The four VDs (VDI to VDIV) and the conserved cysteines are boxed by

solid line. The conserved position where one cysteine is located in all *C. trachomatis* and *C. pneumonitis* MOMP sequences, but where one serine is located in GPIC and Mn MOMPs, is boxed by a broken line. Numbers above boxes denote amino acid residues of serovar E MOMP only.

Paragraph beginning at line 13 of page 12 has been amended as follows:

Any convenient plasmid vector may be used for the MOMP gene or fragment, such as pcDNA3, a eukaryotic II-selectable expression vector (Invitrogen, San Diego, CA, USA), containing a [cyotomegalovirus] cytomegalovirus promoter. The MOMP gene or MOMP gene fragment may be inserted in the vector in any convenient manner. The gene or gene fragments may be amplified from *Chlamydia trachomatic* genomic DNA by PCR using suitable primers and the PCR product cloned into the vector. The MOMP gene-carrying plasmid may be transferred, such as by electroporation, into *E. coli* for replication therein. A MOMP-carrying plasmid, pcDNA3/MOMP, of 6495 bp in size, is shown in Figure 7. Plasmids may be extracted from the *E. coli* in any convenient manner.

Paragraph beginning at line 11 of page 13 has been amended as follows:

The data presented herein also demonstrate the importance of selection of an antigen gene or gene fragment for DNA immunization. The antigen gene elicits immune responses that are capable of stimulating recall immunity following exposure to the natural pathogen. In particular, injection of a DNA expression vector encoding the major outer surface protein (pMOMP) or fragment thereof but not one encoding a cytoplasmic enzyme (CTP synthetase) of *C. trachomatis*, generated significant protective immunity to subsequent chlamydial challenge. The protective immune response appeared to be predominantly mediated by cellular immunity and not by humoral immunity since antibodies elicited by DNA vaccination did not bind to native EBs. In addition, MOMP DNA but not CTP synthetase DNA immunization elicited

cellular immunity readily recalled by native EBs as shown by positive DTH reactions.

Paragraph beginning at line 4 of page 17 has been amended as follows:

Another, possibly more feasible, way is to design a multivalent vaccine based on multiple MOMP genes. The latter approach is justified by the fact that the inferred amino acid sequences of MOMP among related serovars is relatively conserved (see Figures 20A to 20F) and the repertoire of *C. trachomatis* gene variants appears to be finite (ref. 16). As may be seen from the data presented in the Examples below, a partially non-reactive immune response was elicited by the MOMP gene of serovar C of *C. trachomatis* to the MOMP gene of serovar MoPn of *C. trachomatis* (Figures 16 to 19).

Paragraph beginning at line 33 of page 26 has been amended as follows:

IgG2a antibody titers were approximately 10-fold higher than IgG1 antibody titers suggesting that DNA immunization elicited a more dominant T_{H1}-like response. Injection of MOMP DNA as described in Example 2 resulted in the production of serum antibodies to MOMP (Table 2) as detected in an immunoblot assay (Figures 2A and 2B). However, neither CTP synthetase DNA nor MOMP DNA immunized mice produced antibodies that bound to native *C. trachomatis* EBs (Table 1), suggesting that the antibody responses may not to be the dominantly protective mechanism. A comparison of serum IgG subclasses, IgG2a Figures 4A and 4C [Panel] and IgG_1 (Figures 4B and 4D) [Panel] against MOMP protein (Figures 4A and 4B[Panel]) or MoPn (Figures 4C and 4D[Panel]) induced by DNA immunization as described above, is contained in Figures 4A to 4D.

Paragraph beginning at line 17 of page 27 has been amended as follows:

To investigate whether a cell-mediated immune response elicited by MOMP DNA was functionally significant, *in vivo* protective efficacy was evaluated in

mice challenged intranasally with 1×10^3 IFU of *C. trachomatis* MoPn. To provide a measure of Chlamydia-induced morbidity, the loss in body weight was measured over 10 days following challenge with *C. trachomatis* (see Figure 2[, Panel]A). Mice injected with the unmodified vector were used as negative controls and mice immunized with EBs were used as positive controls. Mice immunized with MOMP DNA intranasally maintained a body weight comparable to that observed among EB immunized mice. Mice intramuscularly immunized with MOMP DNA lost body mass but did so at a rate less than the negative control group.

Paragraph beginning at line 32 of page 26 has been amended as follows:

A more direct measure of the effectiveness of DNA vaccination is the ability of mice immunized with MOMP DNA to limit the *in vivo* growth of Chlamydia following a sublethal lung infection. Day 10 post-challenge is the time of peak growth (ref. 13) and was chosen for comparison of lung titers among the various groups of mice. Mice intranasally immunized with MOMP DNA had chlamydial lung titers that were over 1000-fold lower ($\log_{10}$ IFU 1.3 ± 0.3; mean $\pm$ SEM) than those of control mice immunized with the blank vector ($\log_{10}$ IFU 5.0 ± 0.3; $p<0.01$) (see Figure 2[, Panel]B). Mice intramuscularly immunized with MOMP DNA had chlamydial lung titers that were more than 10-fold lower than the unmodified vector group ($p = 0.01$). Mice intranasally immunized with MOMP DNA had significantly lower chlamydial lung titers than mice immunized with MOMP DNA intramuscularly ($\log_{10}$ IFU 1.3 ± 0.8 versus $\log_{10}$ IFU 0.66 ± 0.3 respectively; $p = 0.38$). The substantial difference (2.4 logs) in chlamydial lung titers observed between the intranasally and intramuscularly MOMP DNA immunized mice suggests that mucosal immunization is more efficient at inducing immune responses to accelerate chlamydial clearance in the lung. The lack of protective effect with the unmodified vector control confirms that DNA *per se* was not responsible for the immune response. Moreover, the absence of protective immunity following immunization with CTP synthetase

DNA confirms that the immunity was specific to the MOMP DNA (see Table 1). Figures 5A and 5B shows similar challenge data at a higher challenge dose.

Paragraph beginning at line 10 of page 29 has been amended as follows:

Balb/c mice were immunized in the quadriceps three times at [a] three week intervals with 100 μg of p½MOMP, pCV1, pCV2, pCV3, pCV4 and pCV5 DNA.

Paragraph beginning at line 34 of page 29 has been amended as follows:

Figure 6[, Panel]A shows that p½MOMP immunization evoked a protective immune response to MoPn challenge as measured by change in body weight post infection and by the *in vivo* growth of MoPn in lung tissue day 10 post challenge. The *in vivo* growth among saline treated mice was $\log_{10}$ 5.8 ± 0.21 and among p½MOMP immunized mice was $\log_{10}$ 3.9 ± 0.25, $p<.001$, Figure [2, Panel]6B. As a positive control, mice immunized with heat killed MoPn EBs or recovered from prior infection with MoPn were markedly and equivalently protected against challenged infection ($p<.0001$).

In the Claims:

Claims 1 to 12 and 30 to 32 have been cancelled.